Amendments to the Specification

Please amend the specification as follows:

Please replace the paragraph beginning on page 15, line 15 with the following amended paragraph:

DNA fragments containing the AccI-NcoI region (Fig. 1) were digested with either Nco I, Hinf I or Sau 96I (New England Biolabs). These fragments were end-labeled in reactions of 50 µl containing 50 mM Tris-HCl (pH 7.2), 10 mM $MgCl_2$, 0.1 mM dithiothreitol, 50 µg/ml BSA, 10 µCi α^{32}P dxTP (Amersham--where x represents the correct nucleotide for fill-in), 2 units E. coli DNA polymerase large fragment (New England Biolabs). Following labeling, single-stranded material was prepared by electrophoresis. Samples were denatured in 30% dimethyl sulfoxide, 1 mM EDTA and 0.05% bromophenol blue at 90°C for 2 hr. Samples were chilled and electrophoresed in acrylamide gels in a Bethesda Research Labs apparatus. DNA was detected by autoradiography and isolated by elution into 10 mM Tris-HCl (pH 7.0), 1 mM EDTA. Chemical degradation of DNA for sequence analysis was conducted using standard procedures. Cleavage at guanine (G) residues was conducted by reaction with dimethyl sulfonate at 22°C for 10 min. Cleavage at adenine (A) residues was conducted by 12 min reaction at 90°C in 1.5 M NaOH, 1 mM EDTA. Cleavage at cytosine (C) residues was conducted using hydrazine in 2 M NaCl for 13 min at 22°C. Cleavage at thymine (T) residues was conducted using hydrazine with no added NaCl for 10 min at 22°C. Following cleavage, all reactions were twice precipitated using ethanol and thoroughly dried. All samples were reacted with 1 M piperidine at 90°C for 30 min. Piperidine was removed by evaporation in a Savant ~~speed-vac~~ SPEEDVAC concentrator. Fragments were separated by electrophoresis in acrylamide gels (BRL HO apparatus) in 8 M urea, 50 mM Tris-borate (pH 8.3), 1 mM EDTA. Detection of degraded ladder was by autoradiography using Kodak XAR5 film at -70°C.

Please replace the paragraph beginning on page 17, line 2 with the following amended paragraph:

High molecular weight DNA (6 µg) from tumor MAC117 (see above) was digested with 12 units restriction enzyme EcoRI (New England Biolabs) in a volume of 100 µl for about one hour at 37°C. DNA was obtained by phenol $CHCl_3$ extraction and ethanol precipitation and resuspended in water at a concentration of 0.1 µg/ml. This DNA (0.2 µg) was ligated to λwes λB

arms (Bethesda Research Labs) (1 μg) using T4 DNA Ligase (New England Biolabs) in a total volume of 20 ml [50 mM Tris-HCl pH 7.4, 10 mM $MgCl_2$ 10 mM dithiothreitol, 0.5 mM spermidine, 1 mM ATP]. This mixture of ligated DNAs was packaged into infectious bacteriophage particles using the ~~Packagene~~ PACKAGENE system (Promega Biotec). These particles were used to infect bacteria BNN45 and about 8 X 10^5 individual phage plaques were obtained.

Please replace the paragraph beginning on page 34, line 20 with the following amended paragraph:

This method involves administering to-the patient one of two types of reagent which preferentially binds cells expressing high levels of the protein product of the erbB-related gene described here. These reagents are either antibodies directed against the protein product or a ligand, which is likely to exist because of the homology of the gene to a growth factor receptor. The ligand is isolated by standard techniques using the intrinsic protein kinase activity of the protein product of the erbB-related gene. Extracts of body fluids and cell culture supernatants are incubated with the protein and γ-^{32}PATP. Thus, provided is a method of detecting amplification or increased expression of a MAC117 gene relative to normal human mammary tissue by reacting a body sample from a patient diagnosed with cancer with antibodies having specific binding affinity for a least a portion of the MAC117 protein product. The presence of ligand is inferred by incorporation of ^{32}P into the protein. The ligand is then purified by standard techniques such as ion exchange chromatography, gel permeation chromatography, isoelectric focusing, gel electrophoresis and the like. The natural ligand or antibody is tagged with one or more agents which will cause injury to cells to which they bind. Such tagging systems include incorporation of radioactive or biological toxins. The present discovery of amplification of the erbB-related gene makes it likely that some tumors carry large amounts of the corresponding protein. Hence, the two type-specific agents will bind in larger amounts to the protein present in the body and thus direct the toxic effects of the reagents to these cells.

Please amend the paragraph at page 21, lines 14-23 as follows:

The availability of cloned probes of the MAC117 gene made it possible to investigate its expression in a variety of cell types. The MAC117 probe detected a single 5-kb transcript in A431 cells (Fig. 4). Under the stringent conditions of hybridization utilized, this probe did not detect any

of the three RNA species recognized by EGF receptor complementary DNA. Provided is a method of detecting amplification of a MAC117 gene, wherein the MAC117 gene contains a nucleotide sequence encoding the amino acids encoded by the 423 nucleotides set forth in Figure 1, in mammary tissue from said patient by hybridizing a nucleic acid derived from breast tissue of said patient with a nucleic acid probe of the MAC117 gene, the amplification of said MAC117 gene relative to normal human breast tissue indicating the presence of human mammary carcinoma in said patient. Thus, MAC117 represents a new functional gene within the tyrosine kinase family, closely related to, but distinct from the gene encoding the EGF receptor.

Listing of Claims

Claims 1-57 (Canceled).

58. (Currently amended) A method of diagnosing human mammary carcinoma ~~cancer~~ in a patient comprising:

~~(a)~~ detecting amplification~~, rearrangement or increased expression~~ of a MAC117 gene wherein the MAC117 gene contains a nucleotide sequence encoding the amino acids encoded by the following nucleotides: gtctacatgggtgcttcccattccaggggatgagctacctggaggatgtgcggc tcgtacacagggacttggccgctcggaacgtgctggtcaagagtcccaaccatgtcaaaattacagacttcgggctggctcggctgctgga cattgacgagacagagtaccatgcagatgggggcaaggttaggtgaaggaccaaggagcagaggaggctgggtggagtggtgtctagc ccatgggagaactctgagtggccacctccccacaacacacagttggaggacttcctcttctgccctcccaggtgcccatcaagtggatggc gctggagtccattctccgccggcggttcacccaccagagtgatgtgtggagttatggtgtgtgatgggggggtgttgggaggggtgggtgag gagccatgg,

in [[a]]mammary tissue ~~or tumor cell sample~~ from said patient by hybridizing a nucleic acid derived from [[a]]mammary tissue ~~or tumor cell sample~~ of said patient with a nucleic acid probe of the MAC117 gene, the amplification~~,rearrangement or increased expression~~ of said MAC117 gene relative to normal human mammary tissue indicating the presence of human ~~cancer~~mammary carcinoma in said patient, wherein the nucleic acid derived from the mammary tissue of said patient is DNA or mRNA;~~ or~~

~~(b) detecting abnormal expression of the protein product of the MAC117 gene by reacting a body sample of said patient with antibodies having specific binding affinity for at least a portion of the protein product, the abnormal expression of said protein product of said MAC117 gene indicating the presence of human cancer in said patient~~.

59. (Currently amended) A method of ~~classifying cancers~~ identifying mammary carcinomas that show amplification of a MAC117 gene comprising:

detecting amplification ~~or increased expression~~ of a MAC117 gene relative to normal human mammary tissue, wherein the MAC117 gene contains ~~either~~ a nucleotide sequence encoding the amino acids encoded by the ~~423~~ following nucleotides:

gtctacatgggtgcttcccattccaggggatgagctacctggaggatgtgcggctcgtacacagggacttggccgct
cggaacgtgctggtcaagagtcccaaccatgtcaaaattacagacttcgggctggctcggctgctggacattgacga
gacagagtaccatgcagatgggggcaaggttaggtgaaggaccaaggagcagaggaggctgggtggagtggtgtcta
gcccatgggagaactctgagtggccacctccccacaacacacagttggaggacttcctcttctgccctcccaggtgc

ccatcaagtggatggcgctggagtccattctccgccggcggttcacccaccagagtgatgtgtggagttatggtgtg tgatgggggtgttgggaggggtgggtgaggagccatgg ~~set forth in Figure 1 or the restriction pattern set forth in Figure 5A~~, in [[a]]mammary tissue ~~or tumor sample containing cells~~ from a patient diagnosed with cancer, ~~or abnormal expression of a protein product of said MAC117 gene~~ by reacting ~~a body sample~~mammary tissue from a patient diagnosed with cancer with antibodies having specific binding affinity for a least a portion of the MAC117 protein product, and

~~classifying~~identifying those cancers from patients whose ~~body samples show~~mammary tissue shows amplification ~~or increased expression~~ of said MAC117 gene ~~or abnormal expression of the protein product of said MAC117 gene~~ relative to normal human mammary tissue ~~as being correlated with amplification of the MAC117 gene or increased expression of the protein product of the MAC117 gene~~.

Claim 60 (Canceled).

Claims 61-65 (Not entered).

66. (Cancelled)

67. (Currently Amended) A method of detecting amplification of a MAC117 gene in mammary tissue, the method comprising:

hybridizing a nucleic acid from the mammary tissue with a nucleic acid probe of the MAC117 gene, wherein the MAC117 gene contains a nucleotide sequence encoding the amino acids encoded by the following nucleotides: gtctacatgggtgcttcccattccaggggatgagctacctgg aggatgtgcggctcgtacacagggacttggccgctcggaacgtgctggtcaagagtcccaaccatgtcaaaattacagacttcgggctggc tcggctgctggacattgacgagacagagtaccatgcagatgggggcaaggttaggtgaaggaccaaggagcagaggaggctgggtgga gtggtgtctagcccatgggagaactctgagtggccacctccccacaacacacagttggaggacttcctcttctgccctcccaggtgcccatc aagtggatggcgctggagtccattctccgccggcggttcacccaccagagtgatgtgtggagttatggtgtgtgatgggggtgttgggag gggtgggtgaggagccatgg,

and wherein the nucleic acid from mammary tissue is DNA or mRNA, and

comparing the copy number of the MAC117 gene in the mammary tissue to the copy number of normal human mammary tissue, an increase in copy number relative to normal human mammary tissue indicating amplification of the MAC117 gene.

68. (New) A method of diagnosing human mammary carcinoma in a patient comprising:

(a) detecting increased expression of a MAC117 gene, wherein the MAC117 gene contains a nucleotide sequence encoding the amino acids encoded by the following nucleotides:
gtctacatgggtgcttcccattccaggggatgagctacctggaggatgtgcggctcgtacacagggacttggccgc
tcggaacgtgctggtcaagagtcccaaccatgtcaaaattacagacttcgggctggctcggctgctggacattgacgagacagagtaccat
gcagatgggggcaaggttaggtgaaggaccaaggagcagaggaggctgggtggagtggtgtctagcccatgggagaactctgagtggc
cacctccccacaacacacagttggaggacttcctcttctgccctcccaggtgcccatcaagtggatggcgctggagtccattctccgccggc
ggttcacccaccagagtgatgtgtggagttatggtgtgtgatgggggggtgttgggaggggtgggtgaggagccatgg,
in mammary tissue from said patient by hybridizing an RNA derived from mammary tissue of said patient with a nucleic acid probe of the MAC117 gene, the increased expression of said MAC117 gene relative to normal human mammary tissue indicating the presence of human mammary cancer in said patient; or

(b) detecting increased expression of the protein product of the MAC117 gene, wherein the MAC117 gene contains a nucleotide sequence encoding the amino acids encoded by the following nucleotides: gtctacatgggtgcttcccattccaggggatgagctacctggaggatgtgcggctcgtacacagg
gacttggccgctcggaacgtgctggtcaagagtcccaaccatgtcaaaattacagacttcgggctggctcggctgctggacattgacgaga
cagagtaccatgcagatgggggcaaggttaggtgaaggaccaaggagcagaggaggctgggtggagtggtgtctagcccatgggaga
actctgagtggccacctccccacaacacacagttggaggacttcctcttctgccctcccaggtgcccatcaagtggatggcgctggagtcca
ttctccgccggcggttcacccaccagagtgatgtgtggagttatggtgtgtgatgggggggtgttgggaggggtgggtgaggagccatgg,
by reacting mammary tissue of said patient with antibodies having specific binding affinity for at least a portion of the protein product, the increased expression of said protein product of said MAC117 gene indicating the presence of human mammary carcinoma in said patient.

69. (New) A method of identifying mammary cancers that show increased expression of a MAC117 gene comprising:

detecting increased expression of a MAC117 gene relative to normal human mammary tissue, wherein the MAC117 gene contains a nucleotide sequence encoding the amino acids encoded by the following nucleotides: gtctacatgggtgcttcccattccaggggatgagctacctggaggatgtgcgg
ctcgtacacagggacttggccgctcggaacgtgctggtcaagagtcccaaccatgtcaaaattacagacttcgggctggctcggctgctgg
acattgacgagacagagtaccatgcagatgggggcaaggttaggtgaaggaccaaggagcagaggaggctgggtggagtggtgtctag
cccatgggagaactctgagtggccacctccccacaacacacagttggaggacttcctcttctgccctcccaggtgcccatcaagtggatgg

cgctggagtccattctccgccggcggttcacccaccagagtgatgtgtggagttatggtgtgtgatgggggggtgttgggaggggtgggtga ggagccatgg,

in mammary tissue from a patient diagnosed with cancer by reacting mammary tissue from a patient diagnosed with cancer with antibodies having specific binding affinity for a least a portion of the MAC117 protein product, and

identifying those cancers from patients whose mammary tissue shows increased expression of said MAC117 gene relative to normal human mammary tissue.

70. (New) A method of detecting overexpression of a MAC117 gene in human mammary carcinoma, the method comprising:

(a) hybridizing MAC117 DNA or mRNA from the tissue with a nucleic acid probe of the MAC117 gene, wherein the MAC117 gene contains a nucleotide sequence encoding the amino acids encoded by the following nucleotides: gtctacatgggtgcttcccattccaggggatgagctacctgg

aggatgtgcggctcgtacacagggacttggccgctcggaacgtgctggtcaagagtcccaaccatgtcaaaattacagacttcgggctggc tcggctgctggacattgacgagacagagtaccatgcagatgggggcaaggttaggtgaaggaccaaggagcagaggaggctgggtgga gtggtgtctagcccatgggagaactctgagtggccacctccccacaacacacagttggaggacttcctcttctgccctcccaggtgcccatc aagtggatggcgctggagtccattctccgccggcggttcacccaccagagtgatgtgtggagttatggtgtgtgatgggggggtgttgggag gggtgggtgaggagccatgg,

an increase in MAC117 DNA or mRNA relative to normal mammary tissue indicating overexpression of the MAC117 gene, or

(b) contacting the tissue with antibodies having specific binding affinity for at least a portion of the protein product of a MAC117 gene, an increase in antibody binding relative to normal mammary tissue, indicating overexpression of the MAC117 gene.